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Dopamine Regulation of Cone-Cone Gap Junctions in Ground Squirrel Retina

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Abstract

Cone photoreceptors are electrically coupled such that when the electrical potential in one cell changes, it also changes in adjacent, coupled cells. Phosphorylation of the cone gap junction protein, connexin 36 (Cx36), determines whether a coupling channel is "open" or "closed". Cx36-containing gap junctions are phosphorylated in the open state and dephosphorylated in the closed state. Cone-cone gap junction modulation has not been extensively studied, however, the presence of rod-cone gap junction modulation raises the possibility that the same is true for cone pairs. Pieces of thirteen-lined ground squirrel retina were removed from the eye and the tissue was treated with either the neurotransmitter dopamine or a dopamine antagonist and then labeled with separate antibodies to Cx36 and phosphorylated Cx36. Cone-cone gap junctions were then imaged in the tissue slices and the colocalization of the antibody labels under the two conditions was quantified. We hypothesized that dopamine would also modulate electrical coupling between mammalian cone photoreceptors, which is also mediated by Cx36. Preliminary analysis suggests that the ratio of dephosphorylated to phosphorylated gap junctions is higher in dopamine versus the dopamine antagonist treated tissue. The results are consistent with the idea that dopamine, which is released during the daytime, induces dephosphorylation and thus closes gap junctions between cone cells in the ground squirrel, potentially reducing a source of "neural blur"*.

Introduction

When light enters the eye, it passes through the cornea, iris, lens, and vitreous humor to focus finally onto the retina. The retina is a thin, transparent piece of nervous tissue that contains, on one side, the light-sensitive photoreceptors. Two kinds of photoreceptors function under different conditions: the rods are more sensitive to dim light, while cones function better under more intense illumination. Humans have three types of cone photoreceptors that are sensitive to different wavelengths of light, while ground squirrels only have two. In order for an image on the retina to be processed by the brain, cone photoreceptors first convert incident light into an analog or graded electrical signal that is further processed by other nerve cells in the retina. Small changes in light intensity produce small electrical signals in a cone, which must be distinguished from the random electrical noise that exists in all such cells[3]. Cones are electrically coupled such that when the membrane potential in one cell changes, it also changes in another; in this way, cone-cone coupling reduces the random electrical noise present by allowing the signal to spread and thereby average-out[7]. A point of light incident on the cornea is blurred by the eye's optics so that it typically produces a common response in neighboring cones. Common responses are not attenuated by averaging. The amount of blur increases with pupillary diameter in dimmer lights, and my hypothesis is that the amount of cell-cell coupling should also increase to take advantage of noise reduction without introducing additional signal degradation. Cone-cone coupling is mediated by gap junctions, which are composed of connexin36 (Cx36) proteins[4]. Phosphorylation opens Cx36 channels and increases coupling while dephosphorylation does the opposite. The neurotransmitter dopamine is released by certain retinal neurons in daylight and serves to adapt the retina to function in light. I therefore compared the ratio between the integrated intensity of the pCx36 and Cx36 channels to test whether dopamine would affect the phosphorylation of Cx36 proteins between cone photoreceptors.

Materials and Methods

All procedures were approved by the Northwestern University Animal Care and Use Committee. Thirteen lined ground squirrels (Ictidomys tridecemlineatus, formerly Spermophilus tridecemlineatus) were sacrificed by intracardiac injections of 200 mg/kg of pentobarbital. After extracting the eyes, pieces

** The removal of spatial detail in neural responses, as a result of neural processes rather than optical effects.*

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of the retina were placed vitreal side down on filter paper. The pigment epithelium was removed and the tissue, along with the attached tissue paper, was cut into 100 μm slices. The slices were incubated in either physiological saline containing 100 μM dopamine or dopamine antagonist solution (10 μM each of spiperone and SCH23390) for two hours. The tissue was then fixed with 1% carbodiimide fixative (N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride) in physiological saline for 15 minutes. Tissue was incubated in primary antibodies for Cx36 protein and phosphorylated Cx36 protein for two days at 4°C and then washed with physiological saline solution. Mouse anti-connexin 35/36 was diluted 1:1000 (Chemicon, catalog # MAB3045); the rabbit anti-Cx35 phosphoSer-276 antibody was diluted in 1:1000, which is from Dr. John O'Brien at University of Texas. A glutamate receptor subunit (GluR5) was also labeled with goat anti-GluR5 antibody which was diluted in 1:50 (Santa Cruz Biotechnology, catalog# sc-7616). This was repeated with the secondary antibody using a standard immunostaining protocol. The tissue was mounted onto a microscope slide and imaged using a Zeiss LSM 510 confocal microscope with fixed acquisition parameters. The ratios between phosphorylated and total Cx36 proteins were calculated with Metamorph software. The intensities of the Cx36 and pCx36 were integrated over the connexin plaques and the ratio of pCx36 to Cx36 was found.

Results

A DIC* light image of the photoreceptors shows, photoreceptors and cone terminals indicated with arrows (Figure 1). Immunostaining shows the dopamine treated tissue with the green representing Cx36 protein, red representing pCx36 protein, and yellow representing the overlap. The blue coloring represents the glutamate receptors at the bottom of the photoreceptors (Figure 2A). In comparison, Figure 2B shows dopamine antagonist treated tissue with a similar coloring scheme. Both laser power and photomultiplier gain were held constant when measuring the intensities of Cx36 and pCx36 labeling in control versus treated tissue, so integrating the intensities of each channel in the images would produce comparable ratios of phosphorylation for each image. The normalized ratio of the integrated intensities of the two conditions is shown in Figure 3, and the present data appears to support the hypothesis, showing an 87.09% decrease in phosphorylation from dopamine antagonist to dopamine incubated tissue from six trials.

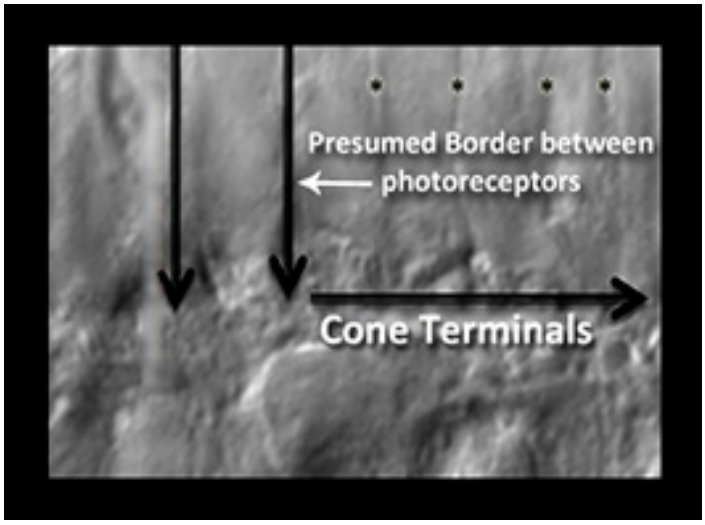

Figure 1. DIC light image of cone photoreceptors.

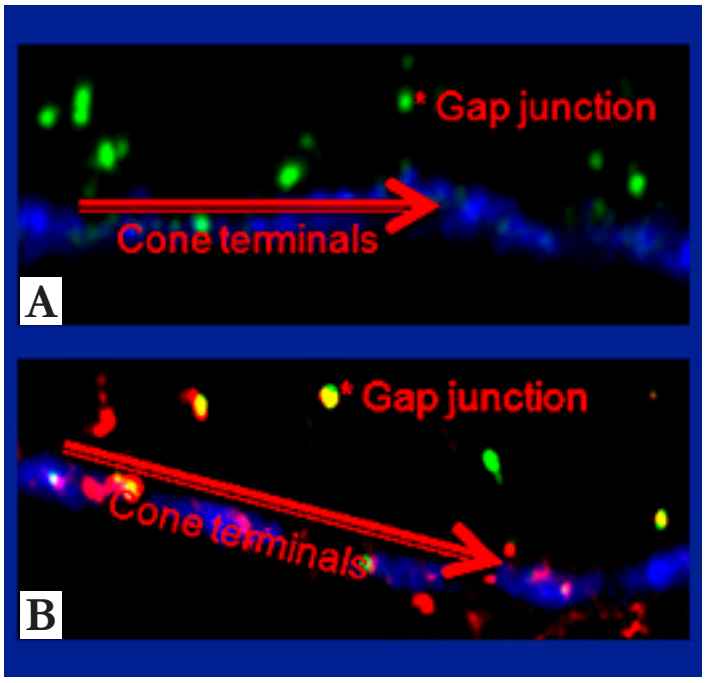

Figure 2. A) Dopamine treated retinal section. **B)** Dopamine antagonist treated retinal section. In both figures, Cx36-containing plaques are green, phosphorylated Cx36 is red, and the combination is yellow. Blue labeling shows the glutamate receptors that are located just below the cone terminals.

** DIC stands for "differential interference contrast microscopy". It is used to create a three dimensional relief image of an object (such as a cell or tissue).*

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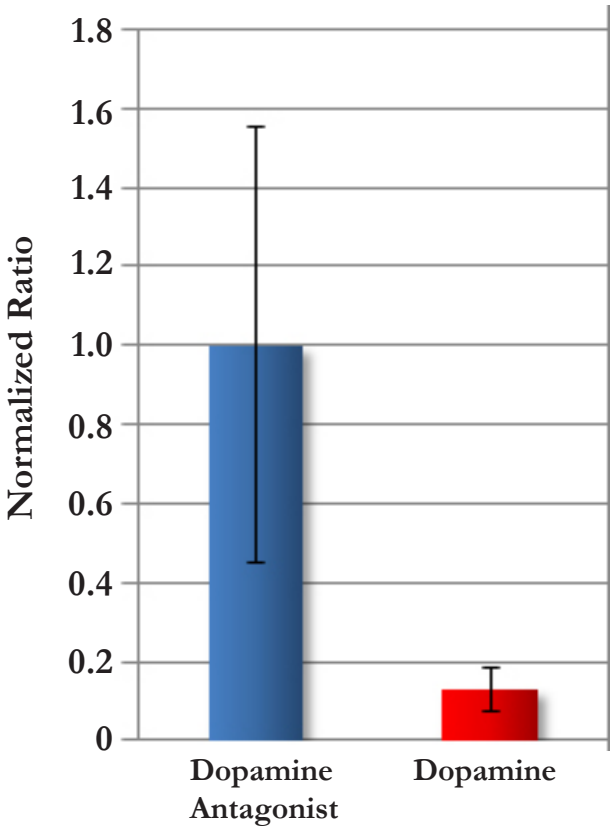

Figure 3. Normalized average ratio of phosphorylated Cx36 to Cx36 from dopamine antagonist and dopamine incubated tissue from six trials. The error bars represent standard deviation.

Discussion

The results show that the amount of pCx36 decreases significantly from the dopamine antagonist to dopamine incubated tissue. Therefore, dopamine reduces the phosphorylation of Cx36. In other preparations, phosphorylated Cx36-containing gap junctions are open and dephosphorylated junctions are closed[6]. Thus, the results imply that dopamine reduces electrical coupling between cones.

Dopamine is released by retinal neurons during the day, and reduces the Cx36-mediated electrical coupling between rod and cone photoreceptors in fish[8]. Our hypothesis was that dopamine would modulate electrical coupling between mammalian cone photoreceptors, since they are also mediated by Cx36. We tested this hypothesis by measuring the effect of dopamine on the phosphorylation of cone Cx36 in ground squirrel retinal slices. Phosphorylated Cx36 gap junctions are open and dephosphorylated junctions are closed[6]. Our results are consistent with the idea that dopamine also closes the gap junction channels between mammalian cones. Cone-cone electrical coupling creates a potentially harmful neural 'blur', but is also beneficial since it reduces electrical noise by averaging the signals in neighboring cones. As long as the blur introduced by the eye's optics exceeds that of the neural blur due to coupling, then the noise reduction due to coupling comes at no cost to vision. The interaction between pupil diameter and optical aberration in the lens and cornea is the main source of optical blur. In bright light, the eye's pupil constricts, and this reduces the blur caused by optical aberrations. The reduced blur potentially causes a mismatch between light spread and coupling, which dopamine may function to counteract by reducing coupling.

Since dopamine levels within the retina are modulated by light, we predict that light should change the phosphorylation state of cone Cx36. This prediction can be tested in future experiments. Other neurotransmitters such as nitric oxide have been shown to modulate gap junction channels in the retina[6]. The effects of these agents can also be tested in future experiments.

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